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# Comparative analysis of phenotypic and molecular diversity in selected pendal and non-pendal genotypes of field bean [*Lablab purpurem* (L.) Sweet]

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**Abstract**

**Twelve field bean (*Lablab purpureus*) genotypes - nine local collections, one check variety of filed bean and one lima bean local type - were selected for the study. The data on 23 quantitative traits revealed significant differences among selected genotypes for many traits. The cluster analysis of genotypes using 23 phenotypic traits revealed 3 clusters. Lima bean formed a separate cluster distinct from field bean genotypes. The pendal and non-pendal (both determinate and semi determinate) types formed two separate clusters within field bean genotypes. The RAPD analysis of 12 genotypes with 20 random decamer primers produced 200 fragments of which 178 were polymorphic. The diversity observed at molecular level was low. The dissimilarity coefficient ranged from 3.23 percent to 11.66 percent among field bean genotypes. Limabean genotype formed a distinct cluster with dissimilarity coefficient ranging from 65.65 to 67.7%. A comparison was made between genetic diversity at phenotypic and molecular level. The genotypes exhibited limited diversity at molecular level and higher diversity at phenotypic level. There was no concurrence between phenotypic and molecular diversity and clustering among the genotypes. Therefore, the degree of genetic diversity appeared to be overestimated if based solely on phenotypic variation in field bean.**

**Key words:** Genetic diversity, RAPD markers, Field bean, Phenotypic variation Introduction

The assessment of the genetic diversity in crop species is of interest for the conservation of genetic resources, broadening of the genetic base and practical applications in breeding. Genetic diversity of a crop species has been investigated using either morphological or molecular markers. Random amplified polymorphic DNA (RAPD) marker [1, 2] has provided powerful tool for the investigation of genetic diversity. Previous studies within *L. purpureus spp.* and sub species using RAPD [3, 4] suggest that there is considerable molecular variation from accessions found in collections that comprise lines from Africa, Asia and Europe. These lines could provide useful and desired traits if incorporated into breeding strategies. In addition to molecular diversity *L. purpureus* also has diverse phenotypic characters [5, 6] reflected in their different growth habits, which have served to provide cultivars suitable to specific environments. The present study was conducted to assess the genetic diversity of different genotypes of *L. purpureus* in local collections using both phenotypic traits and RAPD markers.

## Materials and methods

Seventy-one non-pendal and 43 pendal types collected from different parts of Karnataka were evaluated in two independent experiments during the Kharif season 2007 at Dharwad. Mahalanobies generalized distance ($D^2$) was carried out using the data on 23 quantitative traits, which resulted in 13 clusters in non-pendal and 6 clusters in pendal types suggesting prevalence of diversity in the material (data not given). Five non-pendal genotypes, which included two determinate, and three semi-determinate representing four different clusters and 5 pendal types representing three different clusters were selected to compare the phenotypic and molecular diversity. One lima bean genotype was also added.

### *Phenotypic diversity*

The data collected on 23 quantitative traits *viz*., days to 50% flowering, leaf characters (leaf length, leaf width and leaf let length), number of primary branches per

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plant, inflorescence characters (number of flower buds per raceme, number of racemes per plant, raceme length, peduncle length and number of nodes per raceme), green pod characters (green pod length, green pod width, number of locules per pod, number of seeds per pod), fresh and dry seed characters (seed length, seed width, test weight and shelling percentage) and green pod yield on 12 genotypes were used for phenotypic diversity analysis. The analysis of variance (ANOVA) was carried out for all the 23 traits [7]. The clustering of genotypes based on 23 phenotypic traits was performed using DARWIN software [8].

*Molecular diversity analysis*

*DNA extraction, PCR amplification and electrophoresis*

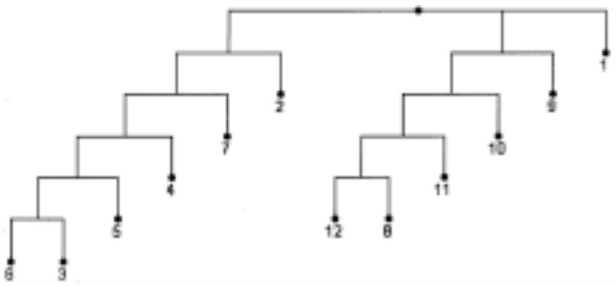
Genomic DNA was extracted from leaf tissues of young seedling by CTBA method [9] with little modifications. The quality and concentration of the DNA was confirmed by electrophoresis on 1% agarose gel. A set of 20 arbitary primers (OPERON technologies, inc. California, USA) was used for amplification of genomic DNA of all the twelve genotypes. The PCR amplifications were carried out in a volume of 20µl consisting of 20-50 ng of template DNA, 5 pm of random decamer primers, 0.1 mM of dNTPs, 1 unit of Taq polumerase (Bangalore Genei, India), I x PCT buffer (10mM tris ph 8.0, 50mM Kill and 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) using Eppendorf master thermal cycler 5331-E. The denaturation was at 95°C for 5 minutes followed by 40 cycles of 1 minute at 94°C, 1 min. at 36°C, 2 min at 72°C and final 8 min extension at 72°C. The electrophoresis of PCR amplified products was carried out on agarose gel of 1.4% in 1X TAB at 80V for 2 hours. The electronic images of ethidium bromide stained gels were captured and documented using UVIdoc (Model: UVIdoc-008-XD)

Only the bands that could be unambiguously scored across all the sampled genotypes were used in this study. The amplified products were scored for each individual as discrete characters. Amplified samples were scored for the presence (1) or absence (0) to create banding matrix of the different RAPD genotypes. The pair wise genetic similarities between genotypes were estimated by DICE similarity coefficient. A cluster analysis based on similarity matrix was performed using the unweighted pair group method with arithmetic averages (UPGMA) using SAHN module.

## Results and discussion

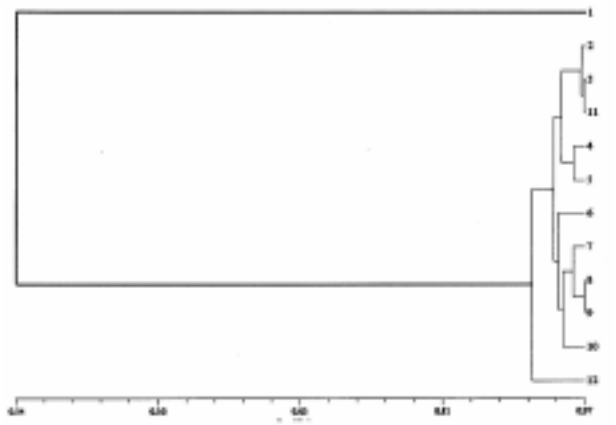
In the present study, twelve genotypes including check HA-3 and one lima bean genotype (DDGC-6) were analyzed for morphological diversity using 23 quantitative variables. The ANOVA suggested that the genotypes differ significantly for days to 50% flowering, number of primary branches per plant, number of flower buds per raceme, number racemes per plant, raceme length, peduncle length, green pod length, number of locules per pod, number of seeds per pod, test weight (fresh seed), shelling percentage (fresh pod), test weight (dry seed), shelling percentage (dry pod), and green pod yield per plant. The genotypic and phenotypic coefficient of variability were also high for green pod yield, test weight (dry), test weight (fresh), green pod length, number of nodes per raceme, peduncle length, raceme length, number of racemes per pant, number of flower buds per raceme, number of primary branches per plant and days to 50% flowering. High variability for many quantitative traits in field bean local collections has also been observed by earlier workers [10, 11, 12]. The genotypes studied represented 3 growth habits - pendal type with indeterminate growth habit, non-pendal semi determinate and non-pendal determinate types. Diverse types were included in the present study representing all 3 growth types. They are also from different geographical origin within the state. The dendrogram constructed from pooled phenotypic data revealed three clusters (Fig. 1). Lima bean was distinct from Dolichos genotypes and formed a separate cluster. Although lima bean resembles pendal types in field bean, it formed a separate cluster revealing its distinctness from Dolichos in many phenotypic traits. The field bean genotypes were grouped into two clusters. The III group consisted of all pendal genotypes such as DDGC-7, DDGC-30, DDGC-33, GL-534, GL-548 and GL-550. It is interesting to note that all the pendal types formed a separate cluster (Cluster III) and the determinate and semi - determinate types were grouped in to another cluster (Cluster II). The growth habit of the field bean genotypes influences many phenotypic traits, which not only distinguish pendal and non-pendal genotypes but also semi-determinate and determinate types. Further the genetic distance values suggest that the determinate types HA-3 and DDGC-46 were more similar to each other than with semi-determinate types in cluster II. Therefore the classification based on phenotypic traits was same as that of based on growth habit.

The diversity based on agro-morphological characters is often misleading and may poorly reflect actual level of genetic diversity. In many species, a better understanding of genetic diversity has been successfully



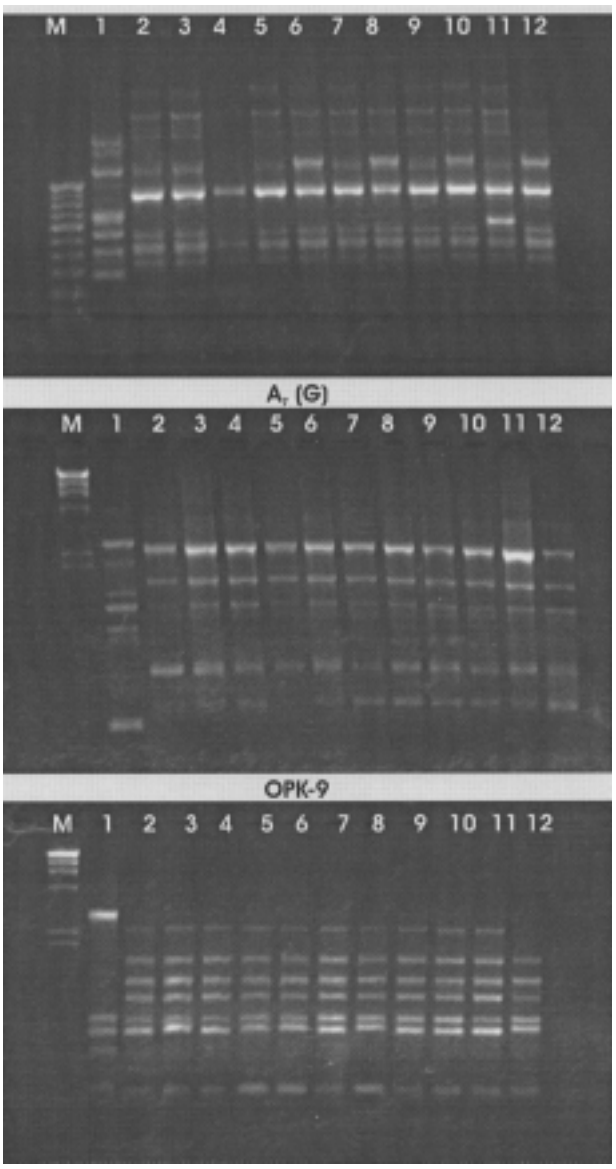
1. DDGC-6 (Lima bean) 2. DDGC-7 3. DDGC-30
4. DDGC-33 5. GL-534 6. GL-5
7. GL-550 8. DDGC-46 9. DDGC-47
10. DDGC-51 11. DDGC-57 12. HA-3 (check)

**Fig. 1. Dendrogram obtained from the pooled data of 23 quantitative variables and 12 genotypes**



1. DDGC-6 (Lima bean) 2. DDGC-7 3. DDGC-30
4. DDGC-33 5. GL-534 6. GL-5
7. GL-550 8. DDGC-46 9. DDGC-47
10. DDGC-51 11. DDGC-57 12. HA-3 (check)

**Fig. 2. Dendrogram obtained from the pooled data of 20 RAPD profiles and 12 genotypes**




1. DDGC-6 (Lima bean) 2. DDGC-7 3. DDGC-30
4. DDGC-33 5. GL-534 6. GL-5
7. GL-550 8. DDGC-46 9. DDGC-47
10. DDGC-51 11. DDGC-57 12. HA-3 (check)

**Fig. 2. RAPD banding pattern in different genotypes of *Lablab purpureus* (L.) sweet along with one lima bean**

achieved in recent times by applying molecular techniques. Phenotypically, *Lablab purpureus* is highly variable [5]. However, little is known about its variation at the DNA level. In the present study, the selected genotypes were analyzed for genetic variation, using random primers and all the primers produced polymorphic bands (Fig. 3). The twenty primers produced a total of 200 reliable fragments. Among these 178 were polymorphic with an average of 89 percent polymorphism (Table 1). The number of bands ranged from 2 (RKAT-6) to 15 (RKAT-20) with an average of 8.9 bands per primer. However, the dissimilarity coefficient determined by pooled data suggested less genetic diversity among the phenotypically distinct types

**Table 1.** Per cent polymorphism in RAPD banding pattern using twenty primers and 11 genotypes of *Lablab purpureus* and one lima bean genotype

| S.No. | Primer name | Number of bands | | | |
|---|---|---|---|---|---|
| | | Total | Poly-morphic | Mono-morphic | %poly-morphic |
| 1 | $A_7$(C) | 8 | 3 | 5 | 37.5 |
| 2 | $A_7$(A) | 12 | 11 | 1 | 91.66 |
| 3 | $A_7$(G) | 13 | 11 | 2 | 84.61 |
| 4 | $A_7$(T) | 11 | 11 | 0 | 100 |
| 5 | OPK-9 | 13 | 12 | 1 | 92.30 |
| 6 | RKAT-2 | 10 | 10 | 0 | 100 |
| 7 | RKAT-4 | 13 | 13 | 0 | 100 |
| 8 | RKAT-5 | 14 | 14 | 0 | 100 |
| 9 | RKAT-6 | 2 | 2 | 0 | 100 |
| 10 | RKAT-8 | 9 | 9 | 0 | 100 |
| 11 | RKAT-12 | 6 | 6 | 0 | 100 |
| 12 | RKAT-14 | 9 | 8 | 1 | 88.88 |
| 13 | RKAT-15 | 9 | 7 | 2 | 77.77 |
| 14 | RKAZ-3 | 7 | 5 | 2 | 71.42 |
| 15 | RKAZ-9 | 11 | 10 | 1 | 90.90 |
| 16 | RKAZ-12 | 13 | 12 | 1 | 92.30 |
| 17 | RKAZ-17 | 11 | 10 | 1 | 90.90 |
| 18 | RKAZ-20 | 15 | 11 | 4 | 73.33 |
| 19 | Oligo 625 | 7 | 6 | 1 | 85.71 |
| 20 | OPC02 | 7 | 7 | 0 | 100 |
| | Total | 200 | 178 | 22 | 89 |



studied. The dissimilarity coefficient ranged from 3.23 per cent to 11.66 per cent among all the field bean genotypes excluding lima bean (Fig. 2). Such limited molecular diversity in the Indian collections of *L. purpureus* particularly from Southern states of India has been reported [13]. Similarly, less polymorphism/ diversity was observed in cultivated germplasm [3], only inclusion of wild accessions resulted in higher molecular diversity. Although phenotypically distinct, diverse field bean genotypes were included in the present study, limited molecular diversity was observed. Probably changes in a few major genes result in the drastic changes in growth habit, concomitantly large scale changes at phenotypic level. The plant growth habit is determined by a single dominant gene [14]. Change at a single locus result in the change in growth habit. The change in growth habit bring in changes in many quantitative traits. High phenotypic variability and low molecular diversity was also reported in world collections [4]. Therefore, the degree of genetic diversity appeared to be overestimated if solely on morphological variation in field bean.

The dendrogram constructed from the pooled data revealed two distinct clusters (Fig. 2). Lima bean formed a separate cluster from field bean suggesting lima bean is distinct from field bean at both genetic and phenotypic level. All the field bean genotypes formed a second cluster. Within second cluster, HA-3 (check) formed separate group which is photo-insensitive, determinate cultivated variety. However, the clusters based on the marker did not group the genotypes as that of based on phenotypic traits or growth habit. The results indicate that there may not be a large number of differences between pendal and non-pendal types at DNA level. Therefore, the markers were not able group the genotypes in to phenotypically intended categories. In addition the markers used for diversity analysis were also limited. It is required to analyze the genotypes with more number of reliable markers to draw any valid conclusions on molecular diversity.

A comparison between morphological and molecular diversity, clearly revealed that there was sufficient phenotypic diversity among the genotypes used for the study. On the other hand, limited molecular diversity was noticed in field bean. The molecular diversity in field bean was not concurrent with morphological diversity [4]. Earlier studies also reported that the extent of diversity in field bean based on morphological traits was different from that based on molecular markers.

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